[Proc. Roy. Soc. Victoria, **46** (N.S.), Pt. II., 1934.]

Art. XVIII.—*The Isolation of the Organism causing Crown Gall on Almond Trees in Victoria.*

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(With Plate IX.)

[Read 14th Deceember, 1933; issued separately 7th May, 1934.]

So far as can be ascertained a pathogenic organism has not definitely been described as the cause of galls on fruit trees in Australia, although such galls are commonly known as Crown Galls and are attributed to *Bacterium tumefaciens* Sm. and T. (1, 3). The object of this note is thus to put on record the isolation and identification of the organism causing Crown Gall in Australia.

Galls found occurring on Almond trees at Eldorado in Victoria were sent to the author by Mr. Adam, of the Victorian Department of Agriculture. Isolation experiments were carried out and an organism isolated in pure culture which, on inoculation into the stems of healthy Almond plants, was capable of producing the typical gall formation.

Distribution of the Crown Gall Disease in Australia.

Although the isolation of the organism from Crown Galls on fruit trees was made from affected Almond trees in Victoria, it may be safely set out that galls occurring on fruit trees in the various Australian States are due to *Bacterium tumefaciens*. Crown Gall has been recorded as occurring in most of the Australian States. It appears particularly to attack Pear and Peach trees in New South Wales(1, 2), and Peach, Pear, Plum, and Almond trees in South Australia(3). It has been found occurring on Almond trees in Victoria and on Hop plants in Tasmania.

Isolation Experiments.

Advantage was taken of the improved technique of isolating the Crown Gall organism as described by Riker(4), and by Robinson and Walkden(5). Fifteen suggestive colonies were selected from the plates resulting from isolation experiments from Almond tree galls; these colonies were subcultured, and after the organisms were found to be in pure culture, inoculations were made into various young and rapidly growing plants.

Five of the cultures used were demonstrated to be virulent, producing quite large galls on a number of hosts, namely, Almond, Peach, Hop, Castor Oil plant (Pl. IX., fig. 1), Tomato (fig. 2), and Sunflower (fig. 3). The same organism was re-isolated in pure culture from the galls which had been experimentally produced on the plants named above.

Description of the Causal Organism.

Morphological Characters.

The organism is a small rod-shaped bacterium, measuring from 0.9 to 1.6μ in length, and 0.4 to 0.5μ in diameter. It occurs singly or in pairs, and is motile by one to two polar flagella. Neither spores nor capsules have been observed. It is Gram negative and not acid fast.

Cultural Characters.

All cultures were incubated at 25° C. unless otherwise noted.

Beef Agar Plates.—Surface colonies develop within 64 hours, and finally reach a size of from three to four millimetres. The colonies are circular, white, at first translucent, later becoming rather opaque with a translucent margin. The edges of the colonies remain regular.

Beef Agar Slope, pH 7.—Growth is moderate, raised, moist, wet-shining, regular to slightly undulate-edged. No odour is produced.

Gelatine Plates.—The plates were incubated at 20° C. Colonies appeared within 64 hours, being small, round, white, and regular-edged. The colonies were translucent at first, later becoming opaque white.

Gelatine Stab.—A whitish, moist, spreading growth occurred at the surface. Growth continued in the depth for two centimetres, and was filiform in character. No liquefaction occurred.

Gelatine Slope.—Growth on gelatine slopes was whitish, wrinkled, somewhat dull, and the edges were irregular.

Beef Bouillon.—The organism grew well in broth, good uniform clouding occurring within 64 hours.

Litmus Milk.—The colour of the litmus milk was reduced by the twelfth day to a greyish white, and slight clearing had occurred. No clot occurred in the milk.

Potato Cylinders.—Abundant, moist, wet-shining, spreading growth occurred on the potato slices. After some days a suggestion of browning became apparent.

Uschinsky's Solution.—Growth in Uschinsky's solution was moderate. A pellicle was formed.

Cohn's Solution.—No visible growth occurred in this medium.

Physiological Characters.

Fermentation Reactions:—

Dextrose Broth.—Good uniform clouding appeared in 64 hours, and a pellicle was formed. Neither acid nor gas was produced.

Lactose Broth.—Uniform clouding with the formation of a thin pellicle occurred in 64 hours. Neither acid nor gas was produced. After some days a whitish deposit gathered at the base of the tube.

Galactose Broth.—Clouding occurred in 64 hours, and later a pellicle was formed. Neither acid nor gas was produced.

Saccharose Broth.—Good growth in 64 hours with the formation of a pellicle. Neither acid nor gas was produced. A whitish deposit gathered at the base of the tube.

Nitrate Reduction.—Nitrates are not reduced. The organism was grown in nitrate broth, the tests for the presence of nitrite being made at the end of five, ten, and fifteen days with sulphanilic acid and alpha-naphthylamine in acetic acid.

Hydrogen Sulphide.—Hydrogen sulphide was produced.

Ammonia Production.—Ammonia was produced.

Indol Production.—Indol was not produced. Peptone water, using Witte's peptone, was employed, and the Ehrlich test was used.

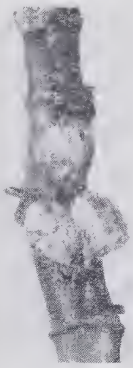
Diastatic Reaction.—No diastatic reaction was observable. Tests were made after five, ten, and fifteen days.

Temperature Relations:—

The optimum temperature for growth was found to lie between 25° C. and 30° C. No growth occurred above 37° C., while a scanty growth was visible when the organism was grown at 1° C.

Thermal Death Point.—Growth of the organism was found still to occur at 51° C., but not at 52° C.

Parallel experiments to the above were made using cultures of *Bacterium tumefaciens* which were kindly forwarded by Dr. N. Brown, of the United States Department of Agriculture. The cultural and physiological characters of the Australian organism were found to agree with those of the type culture in all essential respects. Such differences as were observed, as the inability of the Australian organism to clot milk and the absence of a pellicle and stringing gelatinous threads in broth, do not appear to be significant, since one of the cultures received from America failed to clot milk and the other produced a uniform clouding in broth without the formation of a pellicle. Neither of the type cultures used produced acid in sugar-containing media.



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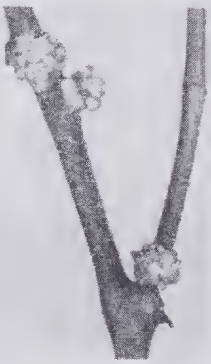

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Crown Galls.

From the above it is concluded that the organism causing galls on fruit trees in Victoria and other States of Australia is identical with *Bacterium tumefaciens* Sm. and T.

In conclusion, the author wishes to express his thanks to Dr. McLennan, of the Botany School, Melbourne, for her helpful advice, and also to Professor Ewart for providing facilities for carrying on the investigation.

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Explanation of Plate IX.

Fig. 1.—Galls produced on Castor Oil plant by *B. tumefaciens* isolated from galls on Almond trees.

Fig. 2.—Galls produced on Tomato plant.

Fig. 3.—Galls produced on Sunflower plant.

Fig. 4.—Galls produced on Castor Oil plant.